REMARKS

Reconsideration and allowance of the subject application is solicited.

In the December 23, 2008 Office Action, claim 1 is rejected under 35 U.S.C. §103(a) as obvious over Blum-Oehler et al (WO 98/44134) in view of Trevors et al. The Examiner cites Blum-Oehler for the disclosure of Nissle 1917 (presumed to be the same as DSM 6601), but acknowledges that this reference does not teach a plasmid-free clone of DSM 6601. However, the Examiner argues that Blum-Oehler teaches that the Nissle 1917 strain has two plasmids (pMut1 and pMut2) that are cryptic and without benefit to the host. The Examiner cites Trevors et al. for teaching methods for removing bacterial plasmids. Combining Blum-Oehler and Trevors, the Examiner has found claim 1 obvious.

In the Office Action, the Examiner noted that in our last Response we asserted that the methods taught by Trevors to cure bacteria of plasmids would not be useful for the particular clone DSM 6601. The Examiner questioned the support for this assertion, and asked whether we are stating that the methods in Trevor simply would not work.

Our assertion actually is that someone having ordinary skill in this art would not have found Trevors sufficiently instructive (and would not have been led) to effectively cure the DSM 6602 bacteria of plasmids pMUT1 and pMUT2 – such that it is "plasmid-free" as claimed. Trevors et al. is a review article that describes several methods for "curing" bacteria from plasmids; however, none of these methods equate to the actual method the inventors used to construct the claimed plasmid-free strains – which method required significantly more than mere routine procedures (see page 2 of our application). Trevors is a general teaching at best, even teaching against the successful curing of plasmids:

> "[T]he usefulness of curing agents is unpredictable in many bacterial strains, as there are no standard protocols applicable to all plasmids" (p.149, c.1, l.25).
>
> "[M]any plasmids can not be cured..." (p.149, c.2, l.15).
>
> "Although all of the plasmid curing agents discussed in this manuscript have been employed to enhance the frequency of plasmid lost, they are only useful against **some** plasmids. Therefore, when initially working with new plasmids and bacterial isolates a **wide variety of curing methods may have to be tried** prior to obtaining a satisfactory method." (emphasis added) (p.155, c.1,

1.15).

Even if we were to assume that the desirability of having a plasmid-free E. coli strain DSM 6601 would have been known, does Trevors et al. make such a strain obvious? Would someone having ordinary skill in this art be motivated with a reasonable expectation of success to try each of the curing methods described in Trevors, especially when Trevors itself states how "unpredictable" the usefulness of these methods are, and "only useful against some plasmids", or even that "many plasmids can not be cured"? We respectfully believe that the only reasonable answer must be "no." Trevors does not describe a predictable solution to the problem of obtaining a plasmid-free DSM 6601 strain. Trevors itself emphasizes the unpredictability of its own methods. This is underscored when our own specification is considered, in particular the details of the special paths taken to generate our clones. (See the Examples section).

Simply stated, Trevors is a general review article that teaches some procedures that are effective for curing some plasmids from some bacteria. A fair reading of it is that it discloses some choices for curing plasmids (e.g., different curing agents, antibiotics, and the like), any one of which could be selected for further research on Blum-Oehler's plasmid-containing DSM 6601. However, Trevors would not have been assessed by someone having ordinary skill in this art as providing a predictable solution to achieve a plasmid-free DSM 6601 using the routine measures it describes.

In summary, combining Blum-Oehler and Trevors could not have lead to our strain of claim 1. Reconsideration of this rejection is respectfully requested.

Claims 2-6 are rejected under 35 U.S.C.§103(a) as obvious over Uraji et al. in view of Blum-Oehler et al (WO 99/44134) in view of Trevors et al., and in view of Alexeyev et al.

Responding to our point in our previous response, that Uraji describes curing techniques only for Agrobacterium and not for E. coli, the examiner said that there is no indication that the Uraji's methods would not work in other bacteria, such as E. coli (also a gram negative bacterium).

Actually, we respectfully note that there is an indication that Uraji's methods would not be expected to apply to other bacteria. In their paper, the authors of Uraji et al.

(who are at least persons having ordinary skill in the art or arguably even more skilled than the average biologist) note specifically that their curing technique "...should also be applicable to **other types of plasmids in *Agrobacterium* groups**..." (see abstract of Uraji et al.). Agrobacterium groups alone are stated as the bacteria for which the curing methods would be expected to work, and a fair reading would not extrapolate (reasonably) that Uraji et al.'s methods would be useful for curing plasmids in non-Agrobacterium bacterial species. It follows then that someone having ordinary skill in the art would not have reasonably expected this curing method to be efficient in much more distantly related species such as *E. coli*, whether gram-negative or not. In fact, if it were the case that the authors believed their methods would be applicable for curing of bacteria from other genera, this most likely would have been stated in the paper, in order to increase the impact of their publication.

Regarding the Alexeyev et al., the Examiner notes that this reference is cited to teach the use of a tetracycline cassette in a plasmid, and is combinable with the other three references to make claims 2-6 obvious. Alexeyev et al. list potential applications for their plasmids with antibiotic-resistance gene cassettes and omega elements (see page 65 in this publication):

> "Besides vector construction and in vitro deletion/insertion mutagenesis, constructs described here can be used:
> (i) for construction of other gene cassettes and omega elements;
> (ii) for mobilization of restriction sites;
> (iii) as a source of restriction sites instead of linkers".

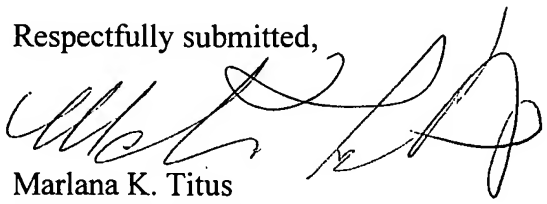
There is no teaching that Alexeyev et al. used antibiotic-resistance gene cassettes for curing of plasmids. In light of this lack of relevant disclosure, someone having ordinary skill in this art, seeking to cure DSM 6601 of plasmids, would not find this reference applicable with any reasonable expectation of success.

It is submitted that someone having ordinary skill in the art would not have found our method of claims 2-6 obvious with any or all of the four cited references in hand. The claimed method for curing *E. coli* strain DSM 6601 from both plasmids is not a technique that is merely a routine combination of the methods in the aforementioned

publications which for an ordinary person skilled in the art would have found obvious. Reconsideration of this rejection is respectfully requested.

In summary, all of the Examiner's outstanding rejections and objections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly solicited. If the Examiner has any questions or would like to make suggestions as to claim language, she is encouraged to contact Marlana K. Titus at (301) 977-7227.

Respectfully submitted,



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